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Review

Radical AdoMet enzymes in complex metal cluster biosynthesis ☆

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ABSTRACT

Radical *S*-adenosylmethionine (AdoMet) enzymes comprise a large superfamily of proteins that engage in a diverse series of biochemical transformations through generation of the highly reactive 5′-deoxyadenosyl radical intermediate. Recent advances into the biosynthesis of unique iron–sulfur (FeS)-containing cofactors such as the H-cluster in [FeFe]-hydrogenase, the FeMo-co in nitrogenase, as well as the iron–guanylylpyridinol (FeGP) cofactor in [Fe]-hydrogenase have implicated new roles for radical AdoMet enzymes in the biosynthesis of complex inorganic cofactors. Radical AdoMet enzymes in conjunction with scaffold proteins engage in modifying ubiquitous FeS precursors into unique clusters, through novel amino acid decomposition and sulfur insertion reactions. The ability of radical AdoMet enzymes to modify common metal centers to unusual metal cofactors may provide important clues into the stepwise evolution of these and other complex bioinorganic catalysts. This article is part of a Special Issue entitled: Radical SAM enzymes and Radical Enzymology.

1. Introduction

The prevalence of reduced iron (Fe)–sulfur (S) mineral phases on early Earth, coupled with the similarity in the structure between FeS minerals and FeS clusters in modern metalloenzymes, prompted the hypothesis that FeS minerals may have been the earliest catalysts on primordial Earth and that these were then incorporated into biology at a point in the distant past [1,2]. This hypothesis is supported by the observation that certain FeS minerals have reactivity toward small molecules. For example, pyrite (FeS_2) catalyzes the reduction of dinitrogen (N_2) to ammonia (NH_3) under conditions mimicking those commonly observed in hydrothermal vent environments [3,4]. Furthermore, sulfur vacancies present on Fe pyrite act to increase the retention time of adsorbed amino acids at the FeS_2–H_2O interface and enhance the reactivity of the Fe and sulfur atoms at the defect site [5–7]. Such observations have led to the notion of "ligand accelerated catalysis" or the modification of FeS clusters with ligand sets that convey distinct reactivity [8,9]. Ligand accelerated catalysis was likely a common process on the early Earth and thus could have played a central role in the generation of the chemical building blocks of life from simple precursors such as the gaseous substrates CO_2, N_2, and H_2 [8–10].

Enzymes that bind FeS clusters are ubiquitous in biology and these metallocofactors perform multiple roles that include the promotion of catalytic and electron transfer functions [11–13]. In addition, FeS cluster containing metalloproteins serve as catalysts in a vast array of chemical transformations that comprise central metabolic reactions and small molecule interconversions [11,14]. The most prevalent FeS clusters observed in biology include [2Fe–2S], [3Fe–4S], and [4Fe–4S] rhombs and cubes. These FeS clusters are typically covalently bound to the protein through cysteine thiolate ligation arising from residues typically organized in conserved motifs [11,12]. More complex FeS centers, such as the FeMo-co found in Mo-nitrogenase and the H-cluster found in [FeFe]-hydrogenase (Fig. 1) display distinct modifications that demarcate them from typical FeS systems. In a manner analogous to FeS mineral surfaces modified with ligands, the chemistry catalyzed by these metalloenyzmes is a direct consequence of the composition, structure, and ligand environment of their active site metallocofactors [15].

While Mo-nitrogenase and [FeFe]-hydrogenase are evolutionarily unrelated, they have developed intriguingly similar pathways for the synthesis and insertion of their respective complex metallocofactors [16]. For example, standard FeS cluster assembly machinery is employed to synthesize the basic building blocks ([4Fe–4S] and [2Fe–2S] clusters) for both the H-cluster and FeMo-co [16]. Modifications are introduced to these standard FeS clusters through the utilization of scaffold (e.g. assembly site) and carrier (e.g. transport) proteins in combination with the activities of radical *S*-adenosylmethionine (AdoMet or SAM) enzymes, culminating in metalloclusters that have enhanced catalytic reactivity and substrate specificity [16]. As will be discussed in greater detail in this review, biochemical and genomic association of radical AdoMet enzymes with scaffold proteins appears to be an emerging theme in complex metallocluster biosynthesis. Herein,

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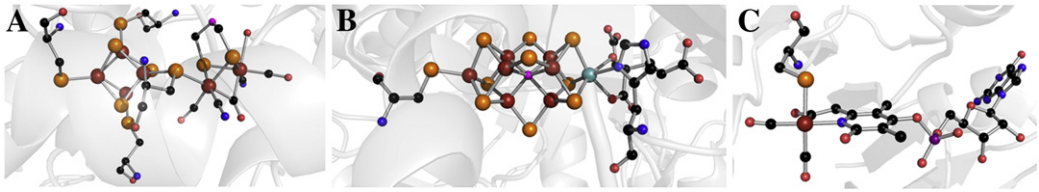

Fig. 1. Examples of complex inorganic cofactors in biology. Crystal structures of the (A) [FeFe]-hydrogenase H-cluster (PDB ID: 3C8Y, 1.39 Å) [22], (B) Mo-nitrogenase FeMo-cofactor (PDB ID: 1M1N, 1.16 Å) [30], and (C) [Fe]-hydrogenase Fe-guanylyl cofactor (FeGP) (PDB ID: 3F47, 1.75 Å) [37]. Atoms are labeled as: carbon (black), oxygen (red), nitrogen (blue), phosphorus (purple), sulfur (orange), iron (rust), molybdenum (cyan), and unknown (magenta).

we detail the unique roles that radical AdoMet enzymes play in the modification of simple FeS clusters in the synthesis of the H-cluster of [FeFe]-hydrogenase and of the FeMo-co of nitrogenase, as well as their role in the synthesis of the iron–guanylylpyridinol (FeGP) cofactor of [Fe]-hydrogenase (Fig. 1). Unifying parallels in the synthesis of these metal cofactors, which may provide important clues into the stepwise evolution of these and other complex bioinorganic catalysts, will be highlighted.

2. Complex metallocofactor structure

2.1. [FeFe]-hydrogenase

[FeFe]-hydrogenases catalyze the reversible reduction of protons to hydrogen ($2H^+ + 2e^- \leftrightarrows H_2$) and contain an active site metal center known as the H-cluster that is comprised of a [4Fe–4S] cubane bridged to a 2Fe subcluster through a cysteine thiolate [17,18]. The coordination environment of the [4Fe–4S] cubane is typical of a FeS cluster, with a single cysteine thiolate ligating each Fe ion. The 2Fe subcluster, however, is unique in biology, comprised of two cyanide (CN^-) and three carbon monoxide (CO) molecules as well as a non-protein bridging dithiolate coordinating the two irons (Fig. 1A). The bridging dithiolate has been reported to be dithiomethylamine [19], although other possibilities including 1,3 propanedithiolate or dithiomethylether have also been proposed [20–23]. The cysteine thiolate that bridges the 2Fe subcluster to the [4Fe–4S] cubane serves as the only covalent linkage between the 2Fe subcluster and the hydrogenase protein. Evidence indicates that this complex bridged cluster assembly is built in stages, with the insertion of the [4Fe–4S] cluster preceding that of the 2Fe subcluster [24]. Indeed, both spectroscopic and structural studies demonstrate that when [FeFe]-hydrogenase is expressed in the absence of maturation proteins, it contains a [4Fe–4S] cluster, presumably assembled by the housekeeping FeS assembly machinery; this [4Fe–4S] cluster is essential for maturation of the protein into an active hydrogenase [25,26]. The structure of the [FeFe]-hydrogenase expressed in the absence of maturation proteins also reveals a positively charged channel leading to the [4Fe–4S] cubane in the active site, presumably providing a pathway for delivery of the anionic 2Fe subcluster that is subsequently closed to form the fully matured [FeFe]-hydrogenase [26].

2.2. Mo-nitrogenase

Mo-nitrogenase catalyzes the reduction of N_2 to NH_3 via the reaction $N_2 + 8H^+ + 8e^- + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16P_i$ by utilizing a bridged, heterometallic MoFeS assembly known as FeMo-co [27,28]. The FeMo-co cluster is a [4Fe–3S] partial cubane that is fused to a [Mo–3Fe–4S] unit through three central, bridging sulfides (Fig. 1B). Each partial cubane cluster is ligated to the nitrogenase protein at the distal metal sites via a His δ amine nitrogen to the Mo ion and a cysteine thiolate to the Fe ion [27]. Additional non-protein ligands to FeMo-co include homocitrate, which binds to the Mo ion [29], and a light atom of unknown identity (most likely either C, N, or O) located at the center of the cluster and coordinating all six Fe ions of central core prismane [30]. Much like the [FeFe]-hydrogenase described above, nitrogenase expressed in the absence of its radical AdoMet maturation enzyme has a cationic channel leading to the FeMo-co active site, and this funnel is closed in the mature enzyme [31].

2.3. Hmd hydrogenase

[Fe]-hydrogenase, or Hmd-hydrogenase catalyzes the heterolytic cleavage of hydrogen, transferring the hydride ion to methenyl-tetrahydromethanopterin to form methylene-tetrahydromethanopterin and a proton ($CH–H_4MPT^+ + H_2 \leftrightarrows CH_2 = H_4MPT + H^+$) [32]. This enzyme is utilized by methanogens that do not synthesize cytochromes to reduce the F_{420} cofactor in methylenetetrahydromethanopterin dehydrogenase [33–35]. The active site of [Fe]-hydrogenase comprises an iron–guanylylpyridinol cofactor (FeGP cofactor) in which the Fe ion is ligated by the nitrogen of the pyridinol ring, the acyl carbon of a substituent of the pyridinol ring, a cysteine thiolate, solvent, and two CO molecules (Fig. 1C) [36,37]. These proteins are universally present in all hydrogenotrophic methanogens, implying their central role in the physiology of these organisms [35].

2.4. Unifying themes

The metal centers at the active sites of Mo-nitrogenase, [FeFe]-hydrogenase, and [Fe]-hydrogenase (Fig. 1) are distinct, and yet have unifying features which include the presence of unusual non-protein ligands (e.g., CO, CN, dithiolate, homocitrate), minimal coordination of the metal cofactor by amino acid residues of the proteins, and the ability to bind and activate diatomic molecules. Further, these unusual features are introduced in part by the activity of radical AdoMet enzymes. The biosyntheses of these three metal cofactors thus reveal fascinating examples of new roles for radical AdoMet chemistry in modifying "typical" metal centers into unusual metal cofactors that serve as efficient catalysts for the interconversions of small molecules such as H_2 and N_2.

3. Radical AdoMet enzymes

The radical AdoMet superfamily was identified by using bioinformatic tools, with the most conserved identifying sequence characteristic being an N-terminal CX_3CX_2C motif [38]. The three cysteines in this motif provide ligands for a site-differentiated [4Fe–4S] cluster essential for catalysis, with the fourth Fe providing a binding site for AdoMet (Fig. 2). Early spectroscopic studies demonstrated that the unique Fe site had distinct properties that were dramatically altered in the presence of AdoMet and revealed the direct coordination of AdoMet to the site-differentiated Fe ion via the amino and carboxyl groups of the methionine portion of AdoMet [39–42]. Spectroscopic, kinetic, and computational experiments have provided support for an inner sphere electron transfer mechanism involving the unique Fe of the reduced $[4Fe–4S]^+$ cluster that promotes homolytic cleavage of AdoMet to yield methionine and the 5′-deoxyadenosyl radical intermediate [40,43–46]. The 5′-deoxyadenosyl radical then abstracts a hydrogen atom from substrate, thereby conveying the ability of radical AdoMet enzymes to catalyze a diversity of chemical reactions that